A NEW SPECIES OF PARAMECIUM (P. MULTIMICRONUCLEATA) EXPERIMENTALLY DETERMINED.[1]

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On September 27, 1909, I received from Dr. Powers two sample cultures of Paramecia with the request that I investigate them as to type and purity of culture. To this end I first killed, fixed, mounted and examined 1,000 individuals. They proved to be neither typical *Paramecium caudatum* nor *Paramecium aurelia*, although most of their characters differed but little from these well-known types. Their length ranged between 144 and 288 μ. Their anterior end was a little blunter and the posterior end a little more pointed than even in *P. caudatum*. The cytoplasm was more dense and more opaque. Their chief difference, however, from hitherto described types of Paramecia lay in the matter of the micronucleus, for, instead of the single micronucleus of *P. caudatum* or the two micronuclei of its variety *P. aurelia*, there is a number of very small bodies, evidently micronuclei, ranging in diameter from about .7 to 1.15 μ (Fig. 3). The characteristic position of micronuclei is fully retained, these bodies lying either in slight grooves or in shallow impocketings of the macronucleus. Like the micronuclei of other types, these delicate bodies are always surrounded by a nuclear membrane.

Of the 1,000 individuals examined 875 distinctly showed from two to six of these small micronuclei, 124 showed apparently no micronuclei whatever, while one appeared at first to possess a micronucleus of the type found in *P. caudatum*. This single instance, however, turned out upon careful study to be a case in which a detached fragment of the macronucleus chanced to simulate in size and appearance the regular micronucleus. As to the 124 which appeared without micronuclei, entire degeneration of these bodies may have been possible, but it is more probable that a slight overstain obscured them, especially when lying behind the macronucleus; the same explanation is doubt-

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less true of the individuals in which but two or three micronuclei were found, others existing but in a less visible location.

Finding thus that the cultures in hand contained nothing but this same type of *Paramecium*, I next proceeded to test the permanence of the type. On October 9, I isolated five individuals, placing each in a clean watch glass containing a definite proportion of sterile and bacterially infected water. These individuals, however, lived but a day. I then again selected five more, varying the proportions of the fluid media. Of these, the two which were placed in water containing the highest percentage of bacteria lived, while the others did not. Three more were started in the same manner as the two successful ones and all these gave living cultures. Of the five living cultures thus obtained two proved much stronger than the others, despite the fact that the conditions were kept as constant as possible in all cases; these two increased rapidly in numbers, while the others increased but little and finally died out after five weeks.

From these two strong cultures, forty individuals were killed, stained and mounted on November 22 and about fifty more on December 17. All of these proved identical in type with the original wild stock. The minute micronuclei were present as before, and again seemed to vary from three to seven in number, which difference depended, in part at least, upon the stain and the transparency of the individual.

Unfortunately during Christmas week extreme cold weather and partial failure of heating plant caused the death of all isolation cultures, the original culture, however, remaining. From this latter, single individuals were again isolated and new cultures started on January 31, the culture media being varied as in previous instances. This resulted again in cultures of varying degrees of strength. One of the best, which I will designate as culture X, was chosen and tested by mounting a number of individuals all of which again proved to be of the multimicronucleate type. This culture X was now accordingly taken as a basis for all further work. From it six cultures were started, the medium being modified in this case by the use of different proportions of agar agar infected with the customary bacteria.

Of these six cultures one, culture Y, was worthy of especial

note in that it produced a few conjugants. Early in April this culture became infected with a minute unicellular alga and, possibly as the result of this, the paramecia became more active and increased more rapidly in number. They also ingested the algæ until they became greenish in color. On April 15 six pairs of conjugants appeared. Three of these were killed in about the three-hour stage of conjugation, another in about the seventeenth hour of conjugation, while the other two pairs were isolated, allowed to complete the act of conjugation, and the ex-conjugants used to start new cultures. It was hoped that stronger cultures would thereby be obtained, but this did not follow. They lived and divided slowly for about three weeks only.

The pairs of conjugants which had been killed were stained and mounted in toto, and are of interest as showing, not only that this type of *Paramecium* is capable of conjugation, but something of the nuclear phenomena undergone during the process. In all cases the micronuclei, or at least a part of them, could be made out. In those killed at the three-hour stage (Fig. 1) all were in pairs, indicating no doubt the customary divisions preceding nuclear exchange. In one case three of these pairs were really single nuclei in advanced division. With difficulty the nuclear membrane could be made out, extending, as in the case of the larger dividing micronuclei of *P. caudatum*, between the separating portions of the dividing nucleus. The micronuclei forming the pairs in these three-hour conjugants were smaller than those in non-conjugants. The macronucleus in this stage is still unchanged except that its surface is more or less furrowed.

In the pair of conjugants killed at the seventeen-hour stage the micronuclei are also present, some again in pairs or in division, some single. The macronucleus on the other hand has now broken up into bands and curved segments, simulating a reticulum. This breaking up of the macronucleus at an early stage does not occur with *P. caudatum*, and, in case further study shows it to be habitual with the present type, this will constitute further proof of its independence.

The limited number of conjugants at our disposal and the consequent inability to procure all the stages have prevented

our demonstration of the actual nuclear exchange during conjugation, but such exchange is naturally to be inferred from the preparatory division of the micronuclei and from subsequent breaking down of the macronucleus. Every feature of the few conjugating pairs examined indicated normal processes and conditions.

Although the cultures started from the ex-conjugants fared so poorly it is worth noting that shortly after the latter were discovered in culture Y this culture underwent a rapid acceleration in growth and division. This may have been due to undetected cases of conjugation occurring in the culture, or on the other hand it may have been the result of some external stimulus, which itself had caused the conjugation. From this rapidly growing culture two further lots of Paramecia have been mounted, and, as before, all prove to be of the multimacronucleate type. Sections have also been made and stained with iron hæmatoxylin (Fig. 3) from different groups mounted during the year. They have fully borne out the results of the more numerous toto mounts.

All told the cultures have been conducted, the first group for three, and the second for five months. They have not been as strong as could be wished, but the best cultures obtained showed at least no tendency to die out and at the close of the work culture Y was multiplying more rapidly than at any previous time. Had our temperature conditions been more uniform and favorable, we should probably have been able to rear much more copious cultures. The entire uniformity of the type throughout these cultures seems good evidence for its permanence and the probability that it deserves specific rank.

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I can fully vouch for the methods and results which my student, Mr. Mitchell, has recorded in the first part of this paper. I may speak a few words further as to my own experience with *P. multimicronucleata*. It is not, in the writer's vicinity, a rare or accidental type. Throughout a number of years of work in eastern Nebraska it has been a frequent and troublesome intruder in my *Paramecium* cultures. The most persistent efforts

have often failed to procure, from wild stock, pure cultures of *P. caudatum*. A portion, usually the bulk, and frequently the whole, of any culture obtained from pond or river water would turn out to be of this multimicronucleate type.

I did not at first recognize the minute micronuclei. I regarded the individuals, which careful and elaborate technique showed to be lacking in the typical micronuclei of *P. caudatum* and *P. aurelia*, as degenerates in the sense of Maupas' contention. As however the hypothesis of the degeneration of the micronucleus became more and more discredited, I reëxamined mounted slides of these Paramecia under high magnifications, with the result that the minute bodies in question were visible in every case. That this type of *Paramecium* was not related to degeneration was further shown by the fact that many pure cultures, unlike those with which Mr. Mitchell has labored so assiduously, have been vigorous and strong growers. I may further mention the fact that in several very large aquaria supplied with running water and a small amount of fresh meat added occasionally, this type of *Paramecium* apparently maintained itself continuously for several years. As often as the organic matter was supplied the animals would multiply and appear in vast swarms in the corners and protected portions of their space; whenever examined they proved of this type and of this type only.

The existence of an undescribed species of *Paramecium* seems improbable. The protozoa are considered of universal distribution, and *Paramecium* is the most-studied genus in existence. Nevertheless much of the study of mircoörganisms is superficial; many have failed to develop a suitable technique, easy as this is, for the certain demonstration of micronuclei; and as to the hypothesis of universal distribution, it is certainly assumed much further than it is proven. Thus, for the last six years, I have made careful search among cultures derived from very numerous wild stocks, for *Paramecium* of the *aurelia* type, *i. e.*, with the well-known two micronuclei. But, aside from a very few isolated individuals derived experimentally from *P. caudatum*, not a single example has been found.

All in all, it seems that, in the light of Mr. Mitchell's results, the type in question deserves specific rank, although this rank

rests upon nuclear differences only. The external characters mentioned by Mr. Mitchell, although holding good for the material investigated by him and for many other lots as well, are not universal, nor have I noted any external character that is. When *P. multimicronucleata* is grown in the same culture with any given strain of *P. caudatum*, the two can usually be readily separated by one or more external characters. Frequently the *P. multimicronucleata* are uniformly larger than the accompanying *P. caudatum*, but they do not exceed the known dimensions of the commoner form, and, in some cultures they are uniformly smaller. So with length of groove, form of ends, opaqueness, etc. The most uniform character that I have seen is that the new type is a little more cylindric in form, bulging less at the point of greatest diameter; but *P. caudatum* approaches this form in starved cultures.

The propriety of basing a species upon nuclear characters only depends of course upon their constancy. Calkins has shown that the types of *Paramecium* with single and double micronuclei respectively are not wholly constant, occasional transitions taking place in both directions. He, therefore, pronounced the types varieties. But the conclusion has again been called in question, the infrequency of the transition leading Kofoid to re-interpret the phenomena as instances of mutation.

I have myself been conducting, during a considerable part of the present year, preliminary experiments on *P. caudatum*, subjecting them to different conditions with a view to ascertaining their possibilities of variation. The only striking results have occurred as the consequences of great changes in feeding habit.

P. caudatum is almost exclusively a bacteria feeder. But as Mr. Mitchell has recorded they occasionally deviate to other minute vegetable organisms. This year I have succeeded in inducing a certain percentage of the individuals from a pure culture of very large and strong growing *P. caudatum* to feed on minor animal organisms, first on flagellates (*Chilomonas*) and then, to a considerable extent, upon smaller ciliates. These very striking changes in food habit produced very striking variations in the Paramecia, both nuclear and cytoplasmic. I will not

describe these at the present time save in so far as they relate to the present discussion. Many of the nuclear changes were erratic and possibly pathological: Macronuclei greatly enlarged, micronuclei unchanged, or sometimes apparently absent, or again enlarged, even more in proportion than the macronucleus, and sometimes divided.

Among the large mass of such material, stained, mounted and examined, I discovered a very few instances of individuals with two typical micronuclei. In fission these micronuclei divided simultaneously and normally. The number of these individuals was very few, probably not exceeding one to several thousand, but they confirm, to some extent, Calkins's observation that *P. aurelia* may arise from *P. caudatum*.

Among the different types of variants I sought assiduously for examples of *P. multimicronucleata*. But none of the exact type were found. Evidently this type is farther separated from *P. caudatum* than is *P. aurelia*. A considerable number of individuals were found however which showed an approach to *P. multimicronucleata*, in that the micronucleus was divided, usually, again, into but two bodies, perfectly normal in appearance, but much smaller than the typical micronuclei of the genus, though a little larger than those of the new type. This variant was one of the most constant and frequent results of the changed diet. In other characters, however, it did resemble closely *P. multimicronucleata* or, for that matter, any recognized type of the genus. I regard it merely as an instance of the well-known law that a powerful stimulus to variation applied to any species brings out, not only new characters, but characters of existing allied species as well. The phenomena, to the writer, serve to confirm, rather than to refute, the specific independence of the new type. But they are of interest in themselves as showing possible lines of experiment leading to nuclear variation. In the present instance it seemed especially worth while to record them, and indeed this is the chief reason for the entire study, in that *Paramecium* is more and more being made the subject of extensive experimental research. So far, little of this study has had regard to other than external characters, but this admitted limitation must soon be remedied, and to this end it is essential that we know the types

of nuclear structure present in the different species or varieties of the genus as well as the lines of variation to which they are subject.

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Explanation of Plate I.

Paramecium multimicronucleata.

Fig. 1. Conjugation near the three-hour stage, showing micronuclei in pairs or in division.

Fig. 2. Conjugation at about seventeen hours. Macronucleus already broken into band-like portions. Micronuclei visible in part, in pairs or single.

Fig. 3. Section of typical *P. multimicronucleata*, an unusual number of the micronuclei chancing to lie in one plane.

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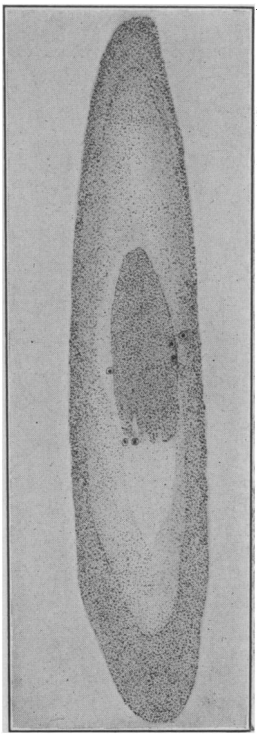

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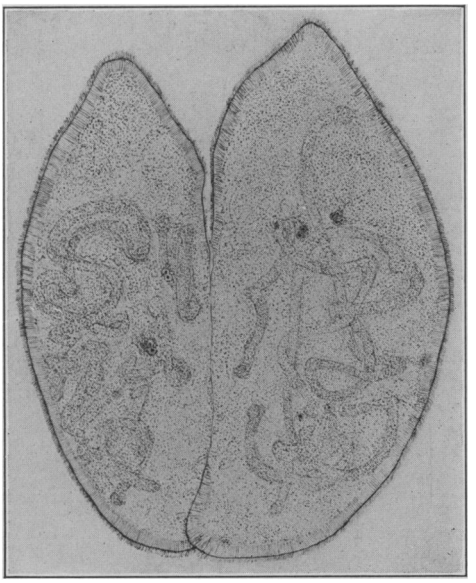

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POWERS AND MITCHELL.